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Photosynthetic responses of turf-forming red macroalgae to high-CO₂ conditions¹

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ABSTRACT

Seaweeds are important components of near-shore ecosystems as primary producers, foundation species and biogeochemical engineers. Seaweed communities are likely to alter under predicted climate change scenarios. We tested the physiological responses of three perennial, turf-building, intertidal rhodophytes, *Mastocarpus stellatus*, *Osmundea pinnatifida*, and the calcified *Ellisolandia elongata*, to elevated pCO₂ over six weeks. Responses varied between these three species.

Ellisolandia elongata was strongly affected by high pCO₂, whereas non-calcified species were not. Elevated pCO₂ did not induce consistent responses of photosynthesis and respiration across these three species. While baseline photophysiology differed significantly between species, we found few clear effects of elevated pCO₂ on this aspect of macroalgal physiology. We found effects of within-species variation in elevated pCO₂ response in *M. stellatus*, but not in the other species. Overall, our data confirms the sensitivity of calcified macroalgae to elevated pCO₂, but we found no evidence suggesting that elevated pCO₂ conditions will have a strong positive or negative impact on photosynthetic parameters in non-calcified macroalgae.

Key words:

macroalgae, photosynthesis, photophysiology, elevated pCO₂, *Ellisolandia*, *Mastocarpus*, *Osmundea*

Abbreviations:

RLC: Rapid Light Curve, F_v/F_m : maximum photochemical quantum yield of PSII; ETR_{max} : maximum electron transport rate; α : initial slope of the ETR vs irradiance function; E_k : minimum saturating irradiance; NPQ_{max} : maximum non-photochemical quenching

INTRODUCTION

Seaweeds are important components of estuaries and coastal waters as primary producers, ecological foundation species, and biogeochemical ecosystem engineers (Jones et al. 1994, Steneck et al. 2002). While their community structure is expected to change in response to future elevated pCO₂ and warming (Brodie et al. 2014), many studies emphasize species- and site-dependence of predicted

macroalgal responses to these pervasive climate stressors (Cornwall et al. 2011, Hepburn et al. 2011, Harley et al. 2012, Cornwall et al. 2013, Koch et al. 2013, Brodie et al. 2014, Cornwall et al. 2017). This makes it difficult to generalize predictions of community change with respect to species' responses, and indeed we see evidence of altered species interactions that can simplify interaction networks and functional assemblages (Kroeker et al. 2013, Teixidó et al. 2018), revise the importance of trophic control in marine macroalgal communities (Falkenberg et al. 2012, 2013, 2014, McCoy and Pfister 2014, Ghedini et al. 2015), or increase species coexistence through greater network intransitivity (McCoy et al. 2016).

Photosynthetic rates are predicted to increase under high-CO₂ conditions due to carbon fertilization in macroalgae and seagrasses, potentially counteracting energetic costs in calcified species or providing an advantage to non-calcified algae (Koch et al. 2013). On the other hand, elevated pCO₂ has broadly caused negative photosynthetic and growth responses in calcified macroalgae, and even reduced survival in many cases (Gao et al. 1993, Hall-Spencer et al. 2008, Ragazzola et al. 2012, Kroeker et al. 2013, McCoy 2013, Comeau et al. 2014, Johnson et al. 2014, McCoy and Kamenos 2015). Carbonate ion availability is reduced in favor of bicarbonate (Doney et al. 2009), which likely increases energetic costs and reduces rates of calcification (Smith and Roth 1979, Gao et al. 1993) that is metabolically linked to photosynthesis (Pentecost 1978).

Changes in future community members of macroalgal-dominated shores thus predict increases in the abundance of small, fleshy macroalgae, which may outcompete larger, slower-growing species under carbon-fertilized future conditions (Harley et al. 2012, Brodie et al. 2014). However, many marine macroalgae utilize bicarbonate by converting it to CO₂ using Carbon Concentrating Mechanisms (CCMs; Raven 1997, Larsson and Axelsson 1999, Cornwall et al. 2011). While CCM use influences the extent to which algae may benefit from elevated pCO₂ (Cornwall et al. 2017), species-specific responses reveal that differences in resource allocation patterns may offset such trends (Kim et al. 2016). For example, down-regulation of CCM use coupled with reallocation of energy to growth versus energy stores may allow species that typically utilize CCMs to outcompete non-CCM users who have been hypothesized to benefit most (van der Loos et al. 2019). In addition to population- (Calosi et al. 2017, Kolzenburg et al. 2019) and species-level (Flynn et al. 2012, McCoy and Ragazzola 2014, Nunes et al. 2015, Vargas et al. 2017) responses to environmental

stress, the effects of individual variation are also important indicators of species' potential for acclimatization and adaptation (Pistevos et al. 2011), yet continue to be understudied as progress is made in documenting community changes and their underlying physiological mechanisms (Demes and Pruitt 2019). Trait variability within a species is an important attribute in itself, as it affects not only a species' ability to survive and specialize in its niche, but its potential for adaptation to new or changing environments (Wiens and Graham 2005, Kawecki 2008). However, while inter-individual variability in stress response can be genetic (Langer et al. 2009), it can also reflect physiological plasticity (Palmer and Strobeck 1992, Gabriel 2005). This has been a focal topic in range shift studies, in which marginal populations may have different plastic regimes and stress tolerances and lower genetic diversity than populations elsewhere in a species range (Kawecki 2008). Intra-individual variability can be defined as the responses of the same individual or clones to different conditions (McManus et al. 1997, Pistevos et al. 2011), or the repeatability of an individual's response to given conditions (Spicer and Gaston 1999).

The concept of within-individual variation can be difficult to study directly in macroalgae due to genotypic variation that has been documented between ramets belonging to a single holdfast, specifically within the Rhodophyta (Santelices et al. 1995, Krueger-Hadfield et al. 2014). This complication impedes traditional studies of intra-individual variability in response to an environmental stress, but can generate useful ecological insights given the natural history of red macroalgae. In this study, we used a mesocosm experiment to simulate elevated pCO₂ conditions on three focal rhodophytes (Rhodophyta) found in similar environments in the North Atlantic mid-intertidal zone: *Ellisolandia elongata*, *Mastocarpus stellatus*, and *Osmundea pinnatifida*. These species are morphologically similar, forming macroalgal turfs on wave-exposed rocky shores, growing in clumps of ramets branching from the same holdfast, or basal crust in the case of *E. elongata*, often co-occurring, and of comparable size. These algal turf-builders are commonly found in mixed assemblages on rocky shores of the United Kingdom and are known to harbor high biodiversity of intertidal invertebrates (Jones et al. 1994). Despite their morphological similarities, *E. elongata* clearly differs from the other two focal species due to its status as a calcifier. *O. pinnatifida*, 'pepper weed,' is known for its secondary metabolites (de Carvalho et al. 2004), which require additional energetic expenditure compared with the relatively undefended *M. stellatus*. CCMs have

been documented in *Corallina* (the former genus of *Ellisolandia elongata* and a close relative), *Mastocarpus*, and *Osmundea* spp. (Stepien et al. 2016), meaning that they may benefit less directly from increased CO₂ availability in seawater (Cornwall et al. 2017). Our experimental manipulations were aimed at testing physiological differences following acclimation to elevated pCO₂ conditions. Additionally, replicate fronds taken from a single holdfast were dispersed among multiple experimental tanks to study the effect of ‘individual’ or clump identity on experimental outcomes.

MATERIALS AND METHODS

Sample collection

Seven clusters of *Ellisolandia elongata*, and *Osmundea pinnatifida*, and eight clusters of *Mastocarpus stellatus*, were collected at low tide from the Plymouth Hoe, from the low-mid intertidal zone on the shore below the Marine Biological Association of the United Kingdom in Plymouth, United Kingdom (50°21’49.3” N, 4°08’28.6” W) on October 26, 2015. All specimens were non-reproductive adults lacking visible sori and were collected from within 3 meters of one another to minimize variation in tidal exposure, microenvironment, and population. Each sampled cluster consisted of multiple ramets, each branching from a single holdfast, in *M. stellatus* and *O. pinnatifida*, and in *E. elongata*, samples were upright, articulated fronds branching from the same basal crust. Specimens were brought back to the Plymouth Marine Laboratory in a bucket of seawater and placed in a tank of aerated seawater within 20 min of collection.

Individual ramets were cut into 6-8 replicate fronds, dependent on size of the individual, with sewing scissors and labelled by source ‘individual’ (ramet) using colored thread (Brown et al. 2011, Flöthe et al. 2014). To test the effects of elevated pCO₂ on individual responses within a community, replicate fronds were dispersed among six 1-m³ experimental exposure tanks with at least one replicate per tank, with additional replicates assigned at random such that some experimental tanks contained duplicate fronds from the same individual. Inclusion of multiple algal specimens within an experimental unit for ecophysiological study is common when using well-mixed, large tanks relative to algal biomass (e.g., Brown et al. 2011, Cornwall et al. 2017, Williams et al. 2018), and including experiments where multiple species are incubated together under these conditions (e.g., Comeau et al. 2014, Kim et al. 2016). Within each tank, macroalgal samples were contained in a 20x30-cm lidless,

large-mesh plastic basket that was neutrally buoyant to keep specimens at consistent depth of ~10 cm. The large mesh size of the basket (1-cm²) together with aquarium pumps positioned within each tank in addition to the tank's recirculating pump - one promoting vertical flow and the other circulating water at the surface - provided non-turbulent water flow over all samples.

Additional fronds belonging to different holdfasts (n=3) of each algal species were collected from the original collection site on November 11, 2015 for immediate oxygen evolution measurements, as described below. These measurements provide context for laboratory stress-responses experienced by the specimens over the course of the study.

Laboratory treatments

Indoor 1-m³ mesocosm tanks were filled with 700 L of seawater collected from the Western Channel Observatory L4 station (50°15.0' N; 4°13.0' W). These tanks were in a temperature-controlled room set to follow average monthly sea surface temperature at the L4 station, which varied from 12.6-14.0°C over the course of the experiment, and water was circulated within the tanks using slow flow pumps. Water changes of 10% tank volume were conducted weekly with freshly collected seawater from L4. Light: dark cycles were set according to natural cycles at the start of the experiment; lights turned on at 07:00 and off at 17:30. At the depth of the samples 10 cm below the surface, PAR was approximately ~25 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (AquaRay GroBeam 1500 LED light tile, measured by a Skye light meter with a PAR quantum sensor). This PAR intensity is comparable with other north-temperate experimental systems, measuring 40-50 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ above the seawater surface, which does not account for rapid light attenuation below the surface (Hoffman et al. 2011, Nunes et al. 2016).

The high pCO₂ treatment of 1000 ppm was created by mixing pure CO₂ gas with air pumped from outdoors and verified using a closed path CO₂ analyzer (Li-Cor 820, USA) and the ambient treatment of 400 ppm simply used air coming from outside (modified from Findlay et al. 2008). Both treatments fluctuated slightly as intended with diurnal and day-to-day variation in outside pCO₂. pCO₂ was monitored and adjusted if needed twice daily (morning and evening) using the CO₂ analyzer and tank pH_{NBS} was recorded daily between 9:00 and 10:00 (NBS Scale, Metrohm 913 pH meter). Over the 6-week experiment, control tanks averaged a daily pH of 8.13 and high pCO₂ tanks

averaged 7.71 (Table 1). Small fluctuations in daily tank pH occurred in both control and high pCO₂ tanks and were likely driven by the natural diurnal variability in pCO₂ in the air sourced from outdoors. Recorded pCO₂ of incoming air was lost due to equipment failure. pH differed significantly between high and low pCO₂ treatments (ANOVA, $F_{1,84}=23.75$, $p<0.001$). To measure dissolved inorganic carbon (DIC) and total alkalinity (TA), 150 mL seawater from each tank were sampled weekly and poisoned with saturated HgCl₂ (6.9% w/w), then refrigerated in the dark prior to measurements using a TA Gran Titration System (AS-ALK2, Apollo SciTech, USA) and a DIC Analyzer (AS-C3, Apollo SciTech, USA). DIC varied by pCO₂ treatment (ANOVA, $F_{1,33}=6.08$, $p=0.019$) but TA remained generally constant (ANOVA, $F_{1,33}=2.95$, $p=0.095$). One anomalously low TA measurement in Tank 1.2 caused increased variability in this tank (Table 1), which was immediately corrected by a water change. Tank was not a significant factor in our analyses of physiological response, and tanks did not differ statistically in their TA values. Tanks were kept uncovered to prevent temperature differences developing between tanks. Salinity averaged 36 PSU throughout the experiment (LF197 combination temperature and salinity probe, WTW, USA; Table 1).

Measurements of growth, oxygen evolution, and photophysiology

Growth of algal fronds was measured at the start (October 26, 2015) and end (November 27, 2015) of the experiment. Each sample was blotted dry and weighed (Sartorius R 200 D analytical balance, DWS Data Weighing Systems Inc., Chicago, IL, USA), and net growth was calculated as the total change in mass over the experimental period in the respective pCO₂ treatments. Relative growth rates were calculated as $(\ln(\text{blotted weight at } t_{\text{final}}) - \ln(\text{blotted weight at } t_0))$ divided by time (32 d).

Photosynthetic and respiration rates and photo-physiology were measured to better understand physiological drivers behind growth responses. Oxygen evolution was measured during laboratory incubations using treatment seawater from each sample's respective pCO₂ treatment level. A subset of 3-5 samples of each species from each tank were measured, with *Ellisolandia elongata* having the fewest replicates due to the reduced survival of that species in the high pCO₂ treatment, between November 27 and December 7, 2015 (*E. elongata* n=15, *Mastocarpus stellatus* n=20, *Osmundea pinnatifida* n=18). Individuals were chosen at random, and replicates of the same individual were

chosen from each of the tanks to enable individual-based comparisons. Specimens were sampled in ~0.025 g pieces cut from the apical meristem of each ramet 2-3 h prior to experimental measurements and placed in a holding bucket filled with appropriate experimental treatment water. For measurement, each specimen was placed in a 4 mL vial filled with treatment seawater and stirred with a magnetic flea. Samples were blotted dry and weighed individually before measurement to normalize oxygen evolution per unit algal biomass. Oxygen (O₂) evolution was measured using a FireSting retractable optical probe (PyroScience, Germany). A FireSting robust temperature probe was also placed in the vial to allow the software to compensate for small temperature changes during O₂ measurements. Photosynthesis versus energy-flux (PE) curves were conducted using an LED light source (Euromex L.E. 5211-230) with a 15V-150W bulb on settings 0-10, which produced PAR of 0, 265, 303, 485, 705, and 1290 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Skye light meter with a PAR quantum sensor). Slopes of O₂ accumulation over 12 minutes in the experimental vial were used to calculate O₂ production and consumption rates at each PAR light level.

Pulse Amplitude Modulated (PAM) fluorometry is a non-invasive technique to measure chlorophyll *a* fluorescence parameters. A MINI-PAM Photosynthesis Yield Analyzer (Walz, Effeltrich, Germany) was used to perform Rapid Light Curves (RLCs) on a subset of macroalgal samples (*Ellisolandia elongata* n=16, *Mastocarpus stellatus* n=24, *Osmundea pinnatifida* n=24) between November 30 and December 4, 2015. Samples were dark-adapted for 15 minutes by using the dark leaf clip (DLC8, Walz) and, additionally, placing them in a bucket of seawater from each sample's respective pCO₂ treatment level with a lid in a dark room for subsequent PAM analysis. Following the dark-adaptation period, basal fluorescence F_o and maximal fluorescence F_m of dark-adapted material were determined with the first saturating light pulse (>40000 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) to obtain the maximum quantum yield (F_v/F_m), where F_v = F_m - F_o (Schreiber et al. 1995). RLC exposed the samples to 8 incremental illumination steps (20 s each) at increasing irradiances (PAR = 25, 76, 157, 249, 375, 508, 748, 1028 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) followed by a saturating light pulse (SP). The SP after each illumination period allowed to measure basal (F) and maximal fluorescence (F_m') in the light-adapted state. Those parameters were used to calculate the effective quantum yield (Y or $\Delta F/F_m'$), where $\Delta F = F_m' - F$. The MINI-PAM was operated using WinControl (Version 2.0, WALZ) software.

Electron transport rate (ETR) at each irradiance level of RLC was calculated according to the formula (Figueroa et al. 2003, Celis-Plá et al. 2014):

$$\text{ETR} = Y \times E_i \times A \times 0.15 \quad [\text{Eq. 1}]$$

where: Y is the effective quantum yield in the light-adapted state; E_i is the incident irradiance ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); A is the absorptance of the sampled algae (Figueroa et al. 2003), which was estimated by using a PAR quantum sensor (Skye Instruments, Wales-UK) and calculated as $A = 1 - T$ (where T = transmittance); and 0.15 is the fraction of photons absorbed by PSII in red macroalgae (Grzyski et al. 1997, Figueroa et al. 2003, Celis-Plá et al. 2014; Table 2). The function ETR vs. PAR was fitted to the model described by Eilers and Peeters (1988). Three parameters were obtained from fitted curves: maximum electron transport rate (ETR_{max}) as an estimator of photosynthetic rates, the initial slope (α) as an estimator of quantum efficiency and the minimum saturating irradiance (E_k) as indicator of acclimation to light (Sagert and Schubert 2000), which was calculated from the intercept between ETR_{max} and α (Ralph and Gademann 2005, Celis-Plá et al. 2014). Finally, Stern-Volmer non-photochemical quenching (NPQ), processes by which photosynthesis is regulated and protected at each irradiance of the RLC, was calculated as: $\text{NPQ} = (F_m - F_m')/F_m'$. Maximum non-photochemical quenching (NPQ_{max}) was obtained after fitting the NPQ vs. irradiance function as for ETR_{max} (Eilers and Peeters 1988).

RESULTS

Growth and survival

Net growth and survival over the experimental period were measured to inform algal fitness in each pCO_2 scenario. The calcified *Ellisolandia elongata* responded strongly to elevated pCO_2 , including decreased survival in high- CO_2 conditions and reduced growth rates. Five ramets of *E. elongata* died in the 1000 ppm treatment, spanning samples from four different ‘individuals’. Individual identity of the specimens did not seem to affect mortality. Anecdotally, ramets from one individual were present in 6 replicates of the elevated pCO_2 treatment. Of these 6, two died and one survived all in the same tank, while two others survived in different tanks. Ramets from that same individual survived in all

three control tanks. *Mastocarpus stellatus* and *Osmundea pinnatifida* exhibited 100% survivorship in all treatments (Fig. 1a).

Exposure to elevated pCO₂ levels decreased net growth in *Ellisolandia elongata* ($F_{1,45}=53$, $p < 0.001$) but had no significant effect on growth in *Mastocarpus stellatus* or in *Osmundea pinnatifida* (Fig. 1b). Relative growth rate also decreased with exposure to high pCO₂ only in *E. elongata* ($F_{1,47}=34.8$, $p < 0.001$), with no significant effects on either *M. stellatus* or *O. pinnatifida*. No species showed an effect of tank or an interaction between tank and treatment. Overall, we found strong effects of elevated pCO₂ on the growth and survival of the calcified alga *E. elongata*, while finding no evidence of effects on these parameters in *M. stellatus* and *O. pinnatifida* (Fig. 1, a and b). Within species, individuals exhibited highly variable responses (Tables S1, S2, S3 in the Supporting Information).

Photosynthetic and dark respiration rates

To investigate mechanisms driving observed differences in growth and survival between *Ellisolandia elongata* and the two non-calcified species, we measured photosynthetic and dark respiration rates as proxies for metabolic function in these species. To do so, we measured oxygen evolution over increasing irradiance, termed photosynthesis – energy flux (PE) curves (Fig. 2, a-i). From these curves, we calculated α , indicative of photosynthetic efficiency, and E_k , minimum saturating irradiance following Jassby and Platt (1976). α did not differ either by species (Fig. 2j; 2-way ANOVA, $F_{2,47}=1.28$, $p=0.29$) or by treatment (Fig. 2j; 2-way ANOVA, $F_{2,47}=0.741$, $p=0.39$). E_k also did not vary either by species (Fig. 2k; 2-way ANOVA, $F_{2,47}=0.862$, $p=0.43$) or by treatment (Fig. 2k; 2-way ANOVA, $F_{2,47}=0.030$, $p=0.86$).

Our statistical analysis focuses on photosynthesis at 485 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, which is the lowest PAR output from our light source that exceeded the saturating irradiance for all three species (360, 327, and 323 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ for *Ellisolandia elongata*, *Mastocarpus stellatus*, and *Osmundea pinnatifida*, respectively). O₂ production differed by species (2-way ANOVA, $F_{2,53}=8.06$, $p<0.001$), but no direct or interactive effect of treatment was observed (2-way ANOVA, all $p>0.1$; Figs. 1d, 2, a-i). Within a species, replicate fronds from the same individual were present across multiple tanks. No effect of treatment or of individual identity were found in *E. elongata* or *M.*

stellatus. However, *O. pinnatifida* showed a consistent effect of individual identity (2-way ANOVA, $F_{4,12}=3.61$, $p=0.037$) on photosynthetic output (Fig. 2, d-f). Given the importance of individual identity, we reanalyzed data for *O. pinnatifida* using a linear mixed effects model, with O_2 production as a fixed effect and individual as a random effect, and found no significant effect of treatment on photosynthetic rate (linear mixed effects model, $F_{2,12}=0.426$, $p=0.68$).

Respiration rates did not vary by species (2-way ANOVA, $F_{2,53}=0.417$, $p=0.66$), but did vary by treatment (2-way ANOVA, $F_{2,53}=9.98$, $p<0.001$), exhibiting different treatment responses by species (2-way ANOVA, interaction term $F_{4,53}=7.45$, $p<0.001$). Respiration in *Mastocarpus stellatus* increased (t-test, $t_{15.8}=2.01$, $p=0.062$) with increased pCO_2 , while other species showed no significant overall trend (Fig. 1d). Interactions between pCO_2 treatment and individual identity differed greatly by species. In *Ellisolandia elongata*, there were no stand-alone effects of pCO_2 treatment (2-way ANOVA, $F_{2,7}=2.91$, $p=0.075$) or of individual identity (2-way ANOVA, $F_{6,7}=1.08$, $p=0.456$), but an interaction between pCO_2 treatment and individual (Fig. 2g-i; 2-way ANOVA, interaction $F_{2,7}=5.53$, $p=0.036$). In *M. stellatus*, there was a strong effect of individual (2-way ANOVA, $F_{2,12}=99.8$, $p<0.001$) but no treatment or interactive effect (Fig. 2, a-c). We thus reanalyzed data for all three species using a linear mixed effects model, with respiration rate as a fixed effect and individual as a fixed random effect. Respiration rate increased at high pCO_2 in *M. stellatus* when accounting for the random effect of individual (linear mixed effects model, $F_{2,12}=69.1$, $p<0.001$), but not in *E. elongata* (linear mixed effects model, $F_{2,12}=2.35$, $p=0.13$). In contrast to no effect on photosynthetic O_2 production response, *Osmundea pinnatifida* showed a strong decline in respiration rate at high pCO_2 , accounting for individual identity (linear mixed effects model, $F_{2,12}=8.76$, $p=0.007$).

The physiological performance of individual replicates was inconsistent across tanks and treatments, with high variation between ramets observed (Table S2). The variability observed in our laboratory cultures was similar to that measured on freshly-collected samples from the field, and is thus not attributed to experimental or laboratory conditions or captivity stress. Overall, we observed no clear effects of pCO_2 treatment on photosynthesis. Whilst significant effects on respiration were observed in *Mastocarpus stellatus* and *Osmundea pinnatifida*, there was no consistent trend across species.

Photophysiology

Lastly, we explored changes in photo-physiology at the species and individual scale to further explain species differences in growth, health, and survival, and the variability we found in metabolic function between species and individuals. Maximum photochemical quantum yield of PSII (F_v/F_m) differed between species (1-way ANOVA, $F_{2,61}=31.15$, $p<0.001$), with mean yield for *Ellisolandia elongata* at 0.51, *Mastocarpus stellatus* at 0.61, and *Osmundea pinnatifida* at 0.63 in control treatments. ETR_{max} , maximum electron transport rate, similarly differed between species (1-way ANOVA, $F_{2,61}=28.85$, $p<0.001$), with mean values of 2.3, 3.0, and 3.6 in control *E. elongata*, *M. stellatus*, and *O. pinnatifida*, respectively. NPQ_{max} , maximum non-photochemical quenching, differed most by species (1-way ANOVA, $F_{2,61}=43.98$, $p<0.001$), with means of 0.47, 0.88, and 0.67 in control *E. elongata*, *M. stellatus*, and *O. pinnatifida*. Minimum saturating irradiance, E_k , did not differ between species (1-way ANOVA, $F_{2,61}=0.399$, $p=0.67$). α , indicative of quantum efficiency of photosynthesis, differed greatly between species (1-way ANOVA, $F_{2,61}=23.34$, $p<0.001$).

None of the three species differed in photosynthetic quantum yield or in minimum saturating irradiance between pCO₂ treatments. ETR_{max} differed only in *Osmundea pinnatifida* (two-sample $t_{21.7}=2.69$, $p=0.013$), with a mean of 3.0 in the control treatment and 2.3 at high CO₂. α also differed by treatment only in *O. pinnatifida* (two-sample $t_{21.0}=3.26$, $p=0.004$), with a mean of 67.3 in the control and 44.54 in the high CO₂ treatment. In *Ellisolandia elongata*, NPQ_{max} differed between high and low pCO₂ treatments (two-sample $t_{13.8}=2.22$, $p=0.043$), with a mean of 0.47 in the control treatment and 0.39 at 1000 ppm CO₂. In *E. elongata*, there was also a positive relationship between growth and ETR_{max} ($F_{1,146}=19$, $p < 0.001$) but no relationship in *Mastocarpus stellatus* and *O. pinnatifida*. No treatment differences were measured in *M. stellatus*.

Within a species, replicate fronds from the same individual were present across multiple tanks. These replicates did not perform consistently across treatments (one-way ANOVA within each species, all $p>0.08$), despite large variation between individuals (Tables S1, S2).

DISCUSSION

Our goal was to compare responses to elevated pCO₂ between species of similar morphology, algal turf-builders commonly found in mixed assemblages on rocky shores of the United Kingdom and

known to harbor high biodiversity of intertidal invertebrates (Jones et al. 1994). CCM use has been documented in all three species (Johnston 1992, Mercado et al. 1998, Ragazzola 2009, Moulin et al. 2011). Despite these similarities, the calcified *Ellisolandia elongata* and chemically-defended *Osmundea pinnatifida* may require additional energetic expenditures compared with the *Mastocarpus stellatus*. These *a priori* differences in species ecophysiology were not clearly related to differences in species response to elevated pCO₂ among non-calcified species, while several effects were found in the calcified *E. elongata*.

Overall, the photosynthetic rates exhibited by all three species in our experiment are similar to rates reported for temperate intertidal species (Bell 1993, Kim et al. 2016, Nunes et al. 2016). Differences in species' photophysiology were found, which supports species differences found between temperate intertidal macroalgae from similar shore heights in the literature (Cabello-Passini et al. 2000, Björk et al. 2004, Williamson et al. 2014). Relatively low photosynthetic and respiration rates, photosynthetic yield, and NPQ_{max} in *Ellisolandia elongata* are consistent with the low productivity of calcified macroalgae (McCoy and Kamenos 2015). *Mastocarpus stellatus* appears to be most photosynthetically efficient overall, exhibiting the greatest PAM-derived α and ETR_{max} of the three species, and a low NPQ_{max} despite similar PAM-derived photosynthetic yield relative to *Osmundea pinnatifida* (Figs. 1, 2).

Reduced growth rates or survival have been found in calcified macroalgae under acidified conditions, globally and across short- and long-term studies (Gao et al. 1993, Hall-Spencer et al. 2008, Gao and Zheng 2010, Hofmann et al. 2011, Ragazzola et al. 2012, Kroeker et al. 2013, McCoy 2013, Comeau et al. 2014, Johnson et al. 2014, McCoy and Kamenos 2015). In our short-term study, elevated pCO₂ reduced growth rate, survival, and NPQ_{max} in *Ellisolandia elongata*, but not in *Mastocarpus stellatus* or *Osmundea pinnatifida* (Fig. 1, a-c), though oxygen production increased by roughly 50% and dark respiration rates declined in *O. pinnatifida*. Reduced growth rate and survival may have large repercussions for reproduction and population and community structure for calcifiers, which are slow-growing relative to their macroalgal competitors (Hofmann et al. 2011), and among whom small changes in growth rate result in a disproportionate change in competitive dominance (McCoy et al. 2014).

Photophysiological mechanisms underlying macroalgal responses to elevated pCO₂ remain poorly understood (Cornwall et al. 2017), with much evidence for species-specific responses (Israel and Hophy 2002, Diaz-Pulido 2011, Bender et al. 2014, Nunes et al. 2015). ETR_{max} is expected to increase with pCO₂ due to carbon fertilization (Björk et al. 2004), and indeed increased ETR_{max} has been a documented response to carbon availability under elevated pCO₂ conditions in the calcified *Corallina officinalis* (Williamson et al. 2014), a close relative of *Ellisolandia elongata* (Hind and Saunders 2013). However, *E. elongata* did not exhibit any ETR_{max} response to pCO₂ in our study. This response is similar to that observed by Hofmann et al. (2011) in *C. officinalis*, which was attributed to light limitation under experimental conditions. Together with decreased ETR_{max} with pCO₂ in *Osmundea pinnatifida*, it is likely that lack of additional light energy required to fully take advantage of carbon fertilization may also be responsible for our failure to observe increased ETR_{max} in this study. On the other hand, we found no effect of pCO₂ treatment on NPQ in any species, which has been found to increase in response to elevated pCO₂ together with low light (Xu and Gao 2012), indicating that our photophysiology results are not wholly consistent with light-limited conditions.

The increased photosynthetic rates and growth predicted for fleshy macroalgae (Koch et al. 2013) was not well supported by our data. Except for slightly increased photosynthetic rates in *Osmundea pinnatifida*, we observed no fertilization effect of pCO₂ on growth, photosynthetic yield or photosynthetic rate across both species. It is possible that light limitation with respect to ability to use abundant DIC in elevated pCO₂ treatments has played a role in limiting the response of both *Ellisolandia elongata* and *O. pinnatifida* (Hofmann et al. 2011). Whether or not increased photosynthetic rate in *O. pinnatifida* is related to the energy expenditures related to strong chemical defenses in this species was not tested here, although chemical defense production in intertidal macroalgae from the same region, including the red alga *Palmaria palmata*, was not affected by elevated pCO₂ in a study by Nunes et al. (2015). Overall, the decoupling of photosynthetic rate from growth and photosynthetic yield indicates that any benefits of pCO₂ fertilization must be relatively small if it does indeed occur for *O. pinnatifida* under these conditions. Upregulation of macroalgal carbon concentration mechanisms likely plays a role in this response mechanism and can lead to variability between and within species (Williamson et al. 2014, Stepien et al. 2016). Similarly, Sarker et al. (2013) observed inconsistent responses to pCO₂ between photophysiological parameters in

Chondrus crispus. In *Mastocarpus stellatus* only, we observed increased respiration rates at elevated $p\text{CO}_2$, indicative of increased metabolic rate that may have offset increases in productivity. However, no observed change in any photophysiological metrics renders this explanation less convincing.

Disparity between our P-I vs. PAM-derived α and E_k metrics likely arises from the fundamental differences between these measurement techniques (Ralph and Gademann 2005). Whereas the longer timesteps of a P-I light curve (here, 12 min) allow an alga's photosynthetic performance to reach steady state conditions, the rapid light curves obtained by PAM fluorometry (20 s) measure the alga's actual and immediate photosynthetic state (Schreiber et al. 1997). Thus, the PAM-derived metrics are more strongly influenced by recent light history (Ralph and Gademann 2005), as evidenced in differences between our α and E_k data for each species (Fig. 2, j and k vs. Fig. 3, m and q). Lower E_k and higher α in PAM-derived metrics were likely primarily influenced by recent light history, while those derived by P-I curves were likely representative of field conditions. We note here that P-I-derived E_k and α were similar between specimens freshly collected from the field and those held in control laboratory conditions (Fig. 2, j and k), which suggests that laboratory light conditions may not have strongly affected PAM-derived metrics.

Strong seasonality has been found in the productivity of temperate macroalgae (Littler and Murray 1977, Littler et al. 1979), including in perennial species such as those studied here, which exhibit higher respiration and photosynthetic rates and reduced ETR_{max} and E_k during the warmer, brighter summer season (Williamson et al. 2014, Egilisdottir et al. 2015). The timing of our study, in the late fall, may reflect a period during which photosynthetic rate is not the most indicative trait of fitness in perennial intertidal macroalgae. Indeed, carbon limitation and thus carbon competition is greatest in the spring when macroalgae have entered the growing season yet low temperatures still favor faster reaction rates of RuBisCO (Ní Longphuirt et al. 2013). The red alga *Chondrus crispus*, found in the same environments as the algae studied here, has been found to increase its growth under acidified conditions only when temperature also increased (Sarker et al. 2013). The autumnal timing of our study thus also coincides with a transitional period of slow temperature decline, which may have reduced algal growth and carbon competition. Thus, a full understanding of carbon use in these macroalgae should include both light and temperature manipulations together with $p\text{CO}_2$, as the

seasonal and diurnal variability in those parameters are likely to regulate carbon availability and demand in coastal systems, and may explain the inconclusive results of this study.

The importance of individual variation within a species was variable and detected with statistical confidence only in the respiration rates of *Mastocarpus stellatus*. The degree to which replicate fronds within a ramet truly represented the same genetic individual may be responsible for our low effect of individual as well as our high variability between individuals. In the red alga *Gracilaria chilensis*, intra-clonal variation can be high within ramets and even with carpospores (Santelices et al. 1995), which can manifest in differences in growth between different portions of the same plant (Santelices 1992, Santelices and Varela 1993). This within-individual variation increases the fitness of a ramet by increasing the likelihood of genet survival and leads to large variation within populations, even those that primarily reproduce clonally (Santelices et al. 1995). Intra-clonal variation has not been studied specifically within *Ellisolandia elongata*, *M. stellatus*, or *Osmundea pinnatifida*, but this process is likely to be ubiquitous among morphologically similar red macroalgae inhabiting similar habitats (Brown et al. 2011, Krueger-Hadfield et al. 2014) and it has been observed in terrestrial plants (Cowart and Graham 1999, Anderson and Agrell 2005, Hulshorf and Swenson 2010).

Overall, we found few effects of elevated pCO₂ on the intertidal, turf-building Rhodophytes *Mastocarpus stellatus* and *Osmundea pinnatifida*, and variable consistency between and within ramets and individuals. In contrast, elevated pCO₂ reduced growth and survival of the calcified, intertidal, turf-building alga *Ellisolandia elongata*. These results are in line with predicted declines in calcified algae like *E. elongata* in favor of non-calcified species (Harley et al. 2012, Brodie et al. 2014). Our results from a short-term study do not take into account potential longer-term acclimatory effects, such as mineralogical changes in calcified algae that have been found in low-pH natural systems (Nash et al. 2012, Ragazzola et al. 2013, McCoy and Ragazzola 2014, Kamenos et al. 2016). Even on this shorter timescale, which should have amplified physiological stress relative to ‘natural’ acidification, we note that some *E. elongata* ramets survived and grew under high pCO₂ conditions, suggesting potential for a positive prognosis for this species over time. Metabolism and photo-physiological traits underlying algal growth did not provide evidence of a clear mechanism behind these responses.

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Table 1. Environmental conditions in experimental tanks, giving tank means and standard deviations during the experimental period.

Tank	pCO ₂	DIC, $\mu\text{mol} \cdot \text{kg}^{-1}$		TA, $\mu\text{mol} \cdot \text{kg}^{-1}$		pH		Temp., °C		Salinity	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
1.2	100	2266.9	14.2	1806.3	1055.8	7.70	0.58	13.03	0.38	36.34	0.20
	0										

2.1	100	2241.6	36.2	2369.0	6.1	7.76	0.48	13.47	0.30	36.04	0.14
	0										
2.3	100	2237.0	24.1	2549.7	168.6	7.68	0.62	13.55	0.22	35.72	0.26
	0										
1.1	400	2237.0	12.3	2441.7	23.4	8.13	0.18	13.02	0.15	36.60	0.24
2.2	400	2253.5	42.8	2376.1	2.2	8.10	0.18	13.22	0.25	36.76	0.39
2.4	400	2261.3	52.1	2491.0	118.9	8.16	0.17	13.50	0.19	36.30	0.29

Table 2. Light absorptance for each study species, giving means and standard deviations between all individuals studied with PAM fluorometry.

Species	n	Mean Absorptance, $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	SD
<i>Ellisolandia elongata</i>	16	0.87	0.075
<i>Mastocarpus stellatus</i>	24	0.96	0.013
<i>Osmundea pinnatifida</i>	24	0.90	0.027

Figure 1. a) Survivorship, b) relative growth rates, and c) oxygen evolution rates at a saturating irradiance of $485 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and D) dark respiration rate ($0 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) from control (400 ppm) and high (1000 ppm) pCO_2 laboratory treatment specimens. *Ellisolandia elongata* (lefthand box), *Mastocarpus stellatus* (middle box), and *Osmundea pinnatifida* (righthand box).

Figure 2. Rate of oxygen produced over PE curves. Panels a, d, and g show data for samples immediately following field collection. Panels b, e, and h show data from laboratory control treatments (400 ppm pCO_2) after 5-6 weeks, and panels c, f, and i show data from high- CO_2 (1000 ppm pCO_2) laboratory treatments after 5-6 weeks. Different colors within a species indicate an individual, and shape indicates replicates of that same individual held in different tanks. Lines show a best fit logarithmic curve ($y=\log(x+1)$, normalized to accommodate $x<0$ within the dataset) across all points for visualization. On the right, α (j) and E_k (k) calculated for P-E curves with colors coded as in Figure 1, *Ellisolandia elongata* in pink, *Mastocarpus stellatus* in black, and *Osmundea pinnatifida* in blue (from left to right) within clustered boxplots.

Figure 3. Photosynthetic characteristics calculated from rapid light curves (RLC). The lefthand column (panels a, e, i, m, and q) contains data aggregated by species, showing *Ellisolandia elongata* in pink (lefthand box), *Mastocarpus stellatus* in black (middle box), and *Osmundea pinnatifida* in blue (righthand box). Within each species, bars of the same color represent replicate samples from the same individual that were dispersed among tanks and treatments. In panels a-d, ETR_{max} is measured in $\mu\text{mol electrons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. In panels m-p, E_k is measured in $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Within a species, relative bar position and color correspond to the same individual sample between panels. Statistics are given in results text.

Table S1. Mean photosynthetic rate, standard deviation (SD) and median absolute deviation (MAD) of specimens separated by tank at PAR 485 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Only specimens surviving to the end of the study are included.

Table S2. Mean photosynthetic rate, standard deviation (SD) and median absolute deviation (MAD) of specimens separated by individual at PAR 485 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Only specimens surviving to the end of the study are included.

Table S3. Mean photosynthetic rate of specimens separated by individual and by tank at PAR 485 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Only specimens surviving to the end of the study are included.





